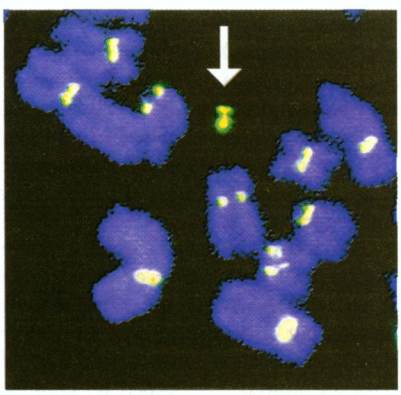
efforts aimed at protecting the natural habitat of the orangutan. Galdikas was instrumental in establishing the largest national park in Borneo, and she is involved with numerous conservation efforts in Indonesia. She has also been involved with the maintenance of a care center for orphaned orangutans and educational programs for children.

In the late 1950s, Schaller conducted the first scientific study of the mountain gorilla, and his book, *The Mountain Gorilla*, led to the establishment of the Virungas National Park in Rwanda. He has also studied tigers, lions, snow leopards, and pandas, and his books are known by field biologists around the world. As director of science for the Wildlife Conservation Society, Schaller has been a leading champion of conservation practices, and his efforts have led to the establishment of national parks in Brazil, East Africa, Mongolia, China, Tibet, and the United States.

Fake DNA

The outlines of chromosome function have been clear for decades, but a new development—the creation of the first artificial human chromosome—gives scientists the tools to fill in the details. Reported by researchers at Cleveland's Case Western Reserve University School of Medicine in the April 1997 issue of *Nature Genetics*, the new chromosome should allow scientists to study gene expression and evolution, and will potentially improve gene therapy.

The finding also provides a model for studying environmental health, says Huntington F. Willard, a genetics professor and senior author on the study. For example, researchers can test how environmental agents cause chromosomes to malfunction during cell division and how toxins cause mutagenesis. Being able to work with an artificial human chromosome brings an experimental dimension to the Human Genome Project, says Willard, whose research has received funding from that project for several years.



John J. Harrington

Small wonder. The synthetic human microchromosome (beneath the arrow) created by investigators at Case Western Reserve University is surrounded by native human chromosomes. Fluorescent dyes cause chromosome material to appear blue while the centromeres appear green.

Artificial chromosomes could correct some defects in approaches to gene therapy as well, the authors contend. Current methods of gene therapy are hampered by unpredictable gene expression and vector shortcomings. For example, viral vectors can foster immune reactions, cause cell toxicity, and transfer only small amounts of genetic material. Available nonviral vectors do not segregate properly during repeated cell divisions.

"We decided the perfect vector would resemble a normal human chromosome," says John J. Harrington, the study's lead author and vice president of Athersys, a Cleveland, Ohio-based company that hopes to develop the new technology. "A micro version small enough to be manipulated and delivered to cells is the optimal way to go." Harrington looks toward a future where physicians can use ready-made chromosomes to treat a variety of genetic diseases.

But such applications are years away from use because of technical challenges, says Uta Francke, a genetics professor at the Stanford University School of Medicine and a member of a National Institutes of Health Panel to Assess the NIH Investment in Research on Gene Therapy, whose 1995 report called for better approaches to gene therapy. Still, Francke said of the new chromosomes, "The applications for biological research are very great."

To create the artificial chromosome, Case Western Reserve researchers pared its structure down to three essential components: centromeres, which guide chromosomes during cell division, telomeres, repeating DNA sequences that protect the ends of chromosomes and allow replication, and origins of replication, the sequences where DNA copying is begun. The scientists cloned centromere sequences consisting of repeats of alpha satellite DNA, huge arrays of a repeating 171 base-pair unit. Previous researchers had been unable to clone these large sequences, and had depended primarily on chopping up existing chromosomes rather than creating new ones. The team then added these centromere sequences, telomere sequences, and genomic DNA digested by enzymes to cultures of human sarcoma cells.

Cells absorbed the genetic material, assisted by positively charged lipids known to aid in DNA uptake. DNA-repair machinery within the cells apparently formed the material into chromosomes in three ways. Two involved hitching the DNA to existing chromosomes, which could disrupt genes. But other cells produced novel chromosomes that resembled naturally occurring ones but were 5–10 times smaller. These microchromosomes replicated normally through six months of mitotic cell division—about 240 generations.

Ethicists expressed optimism that microchromosomes might overcome some disadvantages of gene-therapy vectors, but cautioned that many of the same ethical questions remain. What are the risks of cancer and other side effects? Should adults make such treatment decisions for children? Should new genes be added to germ cells? In addition, questions arise about whether artificial chromosomes could be used to enhance traits such as intelligence, and how to address this possibility, says Michael H. Shapiro of the University of Southern California Law School in Los Angeles. "There are some very serious issues to be discussed," he says.

The Case Western Reserve team built on more than a decade of Willard's work, and on previous studies of yeast genetics. Yeast artificial chromosomes were developed in the early 1980s, and their use has offered insight into gene mapping, function, and identification. But larger, more complex human chromosomes had defied creation until now.

A first step in refining the discovery is ensuring that microchromosomes, not other genetic variants, are formed during the process, and that genes express reliably. Currently, the researchers are building microchromosomes in solution and inserting them into cells in culture to streamline the process. They're planning to inject microchromosomes into mice within the next six months.

The diseases likely to be attacked first with artificial human chromosomes are blood disorders, such as sickle cell anemia. Blood cells are easily removed from the body and reinserted after adding genetic material. Treating other genetic disorders, such as cystic fibrosis, awaits development of new methods for introducing microchromosomes into the body.

A Known Human Carcinogen

A working group of the International Agency for Research on Cancer (IARC), located in Lyon, France, has stated that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the most toxic form of dioxin, is a known human carcinogen. Since 1987, the IARC has classified TCDD as a group B2, or "probable human," carcinogen.

George Lucier, chairman of the IARC dioxin working group, which released its reclassification of TCDD on 11 February 1997, said three lines of evidence were taken into account: animal studies, human epi-

demiological studies, and mechanistic studies. "Just taking the animal and human studies alone probably wouldn't have been enough to classify it as a known human carcinogen," he said.

Until recently, the IARC used only animal and epidemiological studies to support the classification of a compound. The animal studies provided a baseline of where dioxin is active and at what levels it could cause cancer in animals. Coupled with the epidemiological studies, it was shown that those same levels could cause cancer in humans

The human studies went even further to show a positive association between dioxin exposure, dioxin body burden, and cancer risk. This correlation has held up in multiple sites of the body. "The human studies are new and relatively consistent and convincing, but there was still some doubt," said Lucier. "[The characterization] was pushed over the edge by the mechanistic information."

Mechanistic studies showed that TCDD functions like a steroid receptor, although it is distinct from it. One particular receptor, which is required for TCDD to exert its damaging effects, exists in humans as well as in experimental animals. By binding with this receptor, TCDD appears to be able to modulate pathways important in the cell and to change gene expression in these pathways. "All lines of evidence are consistent with the idea that the mechanism by which dioxin causes cancer in rodents is operating in people," said Lucier. The IARC will now use mechanistic information to upgrade or downgrade a chemical's classification.

Because of the change of TCDD's classification to a known human carcinogen, the U.S. EPA will be reevaluating their risk assessment of dioxin and the dioxin models on which they base their regulatory policies. Estimates are that the average body burden of dioxin of approximately 5 parts per trillion in the fat of humans will cause 0–40 cancers per million people over the course of their lifetimes. The EPA is forced to take regulatory action to control exposures whenever a known chemical carcinogen is responsible for greater than one cancer per million.

Dioxin is produced as a by-product of chemical processes in most cases. All of the sources of dioxin pollution have not been identified, which makes it even more difficult to regulate. Lucier hopes to promote the idea of policies to reduce the body burden found in humans, identify the major sources of dioxin, and minimize production from these sources. "Dioxin is symbolic of the clash between environment and industry," said Lucier. "In my mind, the significance of this is to help focus the debate on the dose–response issue where the legitimate debate lies."

EHPnet

More Than Just Unleaded

The environmental effects that have accompanied America's obsession with the automobile are well known. Inefficient internal combustion engines are quickly depleting the world's supply of fossil fuels while emitting pollutants that threaten human health and contribute to global warming. At the same time, U.S. oil imports account for a full quarter of the nation's trade deficit, and this dependence on foreign oil has created numerous foreign policy problems.

However, giving up the freedom that comes with driving in favor of public transportation is not an appealing idea to many car owners, and the 8.5 million new passenger cars purchased in the United States last year are evidence that, despite their drawbacks, motor vehicles remain a fixture of the U.S. culture.

According to information available on the Alternative Fuels Data Center (AFDC) World Wide Web site at http://www.afdc.doe.gov/, however, the solution to this transportation dilemma may be simply to change the way cars are powered. The AFDC is the branch of the U.S. Department of Energy that has been charged with researching and evaluating new technologies to turn the gas-guzzling vehicles that occupy the highways into efficient, environmentally friendly machines.

The Alternative Transportation Fuels and Vehicles link on the AFDC home page is the gateway to a myriad of information on vehicles that run on fuels such as ethanol, methanol, compressed natural gas (CNG), and liquefied petroleum gas (LPG). In many cases, these fuels can be used in the same engines that burn gasoline with only minor modifications. However, they are inherently cleaner than gasoline because they emit less nitrogen oxides and hydrocarbons and because the hydrocarbons they do emit are less likely to react in the atmosphere to form ozone. From the Alternative Transportation Fuels and Vehicles page, the Demonstration & Evaluation Programs page is the hub for links that describe research being conducted on various vehicles including passenger cars, cargo trucks, city buses, and Federal Express delivery vans. For example, fuel economy data are presented for the fleet of the Clean Air Cab Company (under the Light-Duty Vehicle Program link). This Washington, DC-based company is the first taxi service in the United States to operate all of its vehicles on CNG. Reports on the pollution levels associated with each alternative fuel are available under the Emissions Program link on the Demonstration & Evaluation Programs page. Other resources linked to the AFDC home page include maps to alternative fuel refueling sites around the United States, lists of alternative fuel vehicles that are being manufactured for the coming model year, and back issues of two alternative fuel newsletters.

Electricity is the alternative fuel that has the greatest potential for reducing air pollution as battery-powered motors produce no emissions. However, electric cars must be charged frequently and the batteries often have short life spans. These technological limitations combined with the lack of recharging stations in the United States can make owning an electric car a frustrating experience. Until better batteries and a better infrastructure are developed, hybrid electric vehicles (HEVs) may be a more practical technology for reducing air pollution. HEVs combine an electric motor with an engine that burns ethanol, methanol, CNG, or LPG. The combustion engine either assists the electric motor in powering the drive wheels (parallel configuration) or produces electricity to recharge a storage device (series configuration).

Further information about HEVs can be found by following the U.S. DOE Hybrid Electric Vehicle Program link on the AFDC home page. From here, one link takes users to information on the manufacture and sales of HEVs throughout the world, including a look at HEVs included in recent auto shows. Another link allows users to input certain vehicle specifications and run a simulation that calculates the vehicle's performance.

Information about legislation affecting the use of alternative fuels is available under the Alternative Fuel Information link. The two links under the Incentives and Laws section of this page will bring users to a "clickable" map that accesses a list of all the fuel taxes and incentive programs that have been implemented in each state, while a link at the bottom of the page takes users to a glossary of alternative fuel terms. The Biofuels Information Center link on the AFDC page is the door to information on fuels such as alcohols and ethers that can be produced from cellulose-rich biomass such as agricultural products, aquatic plants, and even municipal wastes. For industry or local government leaders who would like to promote the use of cleaner fuels in their areas, the AFDC page provides a link to the U.S. DOE's Clean Cities program. And, if users are unable to locate the information they need on the AFDC site, the home page includes links to a search engine, a site index, and the telephone number of the Alternative Fuels Hotline.